IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:		**Docket No.:**	**4171-102 CIP**
Applicant:	**Jason C.H. Shih**	**Examiner:**	**Zachariah Lucas**
Application No.:	**10/007,613**	**Art Group:**	**1648**
Date Filed:	**October 26, 2001**	**Confirm. No.:**	**4213**
Title:	**METHOD AND COMPOSITION FOR STERILIZING SURGICAL INSTRUMENTS**	**Customer No.:**	**23448**

23448

EXPRESS MAIL CERTIFICATE

I hereby certify that I am mailing the attached documents to the Commissioner for Patents on the date specified, in an envelope addressed to Mail Stop Non-Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, and Express Mailed under the provisions of 37 CFR 1.10.

Candace White

April 16, 2004
Date

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AFFIDAVIT UNDER 37 C.F.R. §1.132 OF DR. JASON C.H. SHIH IN U.S. PATENT APPLICATION NO. 10/007,613

Mail Stop Non-Fee Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, **DR. JASON C.H. SHIH**, being duly sworn, depose and say:

(1) THAT I am the inventor and applicant for U.S. Patent Application No. 10/007,613, filed on October 26, 2001 in the U.S. Patent and Trademark Office in my name for "**METHOD AND COMPOSITION FOR STERILIZING SURGICAL INSTRUMENTS**" (hereinafter "the Application").

(2) THAT the Application discloses and claims a method and a system for disinfecting articles that are susceptible to contamination by infectious prion protein, wherein the articles to be disinfected is first heated to a sufficient temperature and for sufficient time to enhance the proteolytic susceptibility of infectious prion protein associated therewith, and then exposed to a proteolytic enzyme that is effective for at least partial reduction of the infectious prior protein (hereinafter "the Invention").

(3) THAT at my direction, experiments have been conducted to show the efficacy of various proteolytic enzymes in destructing or at least partially reducing infective prion protein in tissue samples at various concentrations with various pre-heating temperatures, according to the following experimental procedure:

> Eight (8) different solutions containing proteolytic enzymes at different concentrations were used to effect degradation and reduction of infectious prion protein responsible for Chronic Wasting Disease ("CWD") in deer and elk, as contained in infected deer brain tissues, after such infected tissues had been pre-heated to different temperatures. The Bio-Rad CWD Antigen Test Kit (Bio-Rad Laboratories at Hercules, CA) was used to determine the presence and concentration of the infectious prion protein in each sample after heat and enzyme treatment.

(4) THAT the above-mentioned eight proteolytic enzyme solutions included:

(a) a solution (A) containing no proteolytic enzyme, in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine;

(b) a solution (B) containing purified PWD-1 keratinase ("KE") to yield a final concentration of about 50 mg/L in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine;

(c) a solution (C) containing KE to yield a final concentration of about 100 mg/L in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine;

(d) a solution (D) containing KE to yield a final concentration of about 200 mg/L in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine;

(e) a solution (E) containing Subtilisin ("PS"), to yield a final concentration of 200 mg/L in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine;

(f) a solution (F) containing crude PWD-1 keratinase ("CKE") to yield a final concentration of about 50 mg/L, together in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine;

(g) a solution (G) containing crude PWD-1 keratinase ("CKE") to yield a final concentration of about 100 mg/L, together in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine; and

(h) a solution (H) containing crude PWD-1 keratinase ("CKE") to yield a final concentration of about 200 mg/L, together in 50mM phosphate buffer, pH 8.0 and 2% N-lauroylsarcosine.

(5) THAT .350 g of CWD-positive deer brain stem tissue was mixed and homogenized in 1.5 mL of homogenization buffer, which was then separated into .500 mL aliquots and pre-heated for 40 minutes at a pre-heating temperature selected from 50°C, 80°C, 90°C, 100°C and 115°C. Samples subject to pre-heating at 115°C were pre-heated for 5 minutes only. Subsequently, the pre-heated tissue sample was

cooled and then treated with the respective proteolytic enzyme solution to yield the appropriate final concentration of enzyme. The pre-heating and enzyme treatment cycle was repeated for each of the eight different proteolytic enzyme solutions with each of the five different pre-heating temperatures. Enzymatic digestion was carried out according to the following:

(1) for KE: digestion at 50°C for 1 hour;

(2) for PS: digestion at 50°C for 1 hour; and

(3) for CKE: digestion at 50°C for 1 hour.

Each enzymatic digestion reaction was stopped by heating for 5 min at 80°C.

(6) THAT four (4) control samples were employed, which included:

(i) 500 µl BioRad Negative Control, (internal negative control provided in the testing kit);

(ii) 500 µl BioRad Positive Control, (internal positive control provided in the testing kit);

(iii) 500 µl Tissue Negative Control, (CWD-negative deer brain stem tissue); and

(iv) 500 µl Tissue Positive Control, (CWD-postive deer brain stem tissue).

(7) THAT all tissue samples and control samples were then examined by enzyme-linked immunosorbent assay (ELISA), according instructions provided in the Bio-Rad CWD Antigen Test Kit.

(8) THAT all the above-described tests, including both the sample tests and control tests, were carried out twice, and the results of which were correspondingly numbered as PLATE 1 and PLATE 2.

(9) THAT the ELISA test results were as follows:

PLATE 1

Lot No.	Protease	Conc.(mg/L)	Temperatures (°C)					Control	
			50	80	90	100	115	Negative	Positive
A			2.606	2.825	2.328	0.386	0.249	0.020	
B	KE	50	2.136	3.012	1.289	0.167	0.107	0.021	
C	KE	100	2.000	2.589	1.146	0.059	0.156	0.020	
D	KE	200	2.527	1.851	0.579	0.046	0.086	0.021	
E	PS	200	0.411	0.364	0.095	0.026	0.107		1.180
F	CKE	50	1.588	0.431	0.427	0.052	0.102		1.161
G	CKE	100	1.226	0.678	0.269	0.049	0.052	0.023	
H	CKE	200	0.987	0.802	0.111	0.060	0.043		3.340

PLATE 2

Lot No.	Protease	Conc.(mg/L)	Temperatures (°C)					Control	
			50	80	90	100	115	Negative	Positive
A			3.311	3.313	3.310	0.265	0.067	0.020	
B	KE	50	3.162	2.599	2.507	0.224	0.032	0.024	
C	KE	100	2.348	1.733	1.169	0.170	0.058	0.021	
D	KE	200	1.881	1.512	0.585	0.060	0.119	0.022	
E	PS	200	0.558	0.261	0.149	0.035	0.065		1.185
F	CKE	50	1.072	0.994	0.331	0.051	0.066		1.175
G	CKE	100	0.810	0.547	0.355	0.042	0.028	0.023	
H	CKE	200	0.516	0.324	0.538	0.062	0.046		3.358

The Bio-Rad negative control test results are provided in rows A-D. The Bio-Rad positive control test results are provided in rows E-F. The tissue negative control test results are provided in row G, and the tissue positive control test results are provided in row H.

(11) THAT the above-tabulated test results evidenced destruction or at least reduction of infectious prion protein in deer brain stem tissue samples initially containing same (sample test results shown in the middle), when such samples were treated by preheating followed by enzymatic digestion, in accordance with the Invention, using enzyme species including keratinase and subtilisin at different pre-heating temperatures ranging from about 50°C to about 115°C.

(12) THAT the prion concentrations in the tissue samples were found to decrease in general with the increase in the pre-heating temperatures, when the same type of proteolytic enzyme species and the same enzyme concentration were used for enzymatic digestion. However, the decrease in prion concentrations leveled off when the pre-heating temperature increased from 100°C to 115°C, and in some cases the prion concentrations even increased, which indicates that pre-heating temperature achieves optimal results at about 100°C.

(13) THAT the prion concentrations in the tissue samples were found to decrease in general with the increase in the concentrations of enzyme solutions used for enzymatic digestion, when the same type of proteolytic enzyme species and the same pre-heating temperature were employed. At the same enzyme concentration, subtilisin and crude PWD-1 keratinase enzymes were both found to be more effective in reducing or destructing infectious CWD prion agent than the purified PWD-1 keratinase.

(14) THAT the 200 mg/L subtilisin solution was effective in destructing from about 83% (as in PLATE 2) to about 84% (as in PLATE 1) of the infectious prion protein contained in a tissue sample that was preheated at a temperature of about 50°C.

(15) THAT the 200 mg/L crude PWD-1 keratinase solution was effective in destructing from about 62% (as in PLATE 1) to about 84% (as in PLATE 2) of the infectious prion protein contained in a tissue sample that was preheated at a temperature of about 50°C.

(16) THAT the 200 mg/L purified PWD-1 keratinase solution was effective in destructing from about 75% (as in PLATE 1) to about 82% (as in PLATE 2) of the infectious prion protein contained in a tissue sample that was preheated at a temperature of about 90°C.

Dr. Jason C.H. Shih

4/16/2004
Date

Dr. Jason C.H. Shih appeared before me on this 16th day of April, 2004. He declared to me that he is the person described in this Affidavit, and he executed this Affidavit before me, and declared that his execution was completely voluntary.

State of North Carolina
County of Wake

NOTARY PUBLIC
My Comm. Exp.
(SEAL)

Paulette R Jaeger
Notary Public

My commission expires: 11/10/08